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TARGETING TOMATO FRUIT ALLERGENS WITH CRISPR/CAS9 TECHNOLOGY

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Tomato is the main fruit and vegetable crop produced worldwide and is considered an important part of the Mediterranean diet. Tomato fruit is rich in nutrients and contains various health-related compounds such as iron (Fe), calcium (Ca), vitamins, antioxidants, polyphenols, and carotenoids. Despite the health benefits that tomatoes provide, some consumers must avoid them in their diet, due to the risk of allergic reactions after their consumption. In fact, tomato has been confirmed as one of the most prevalent allergenic vegetables.

In this work, a genome editing technology known as CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR- associated protein 9) was used to improve the qualitative aspect of tomato by reducing the level of allergenic proteins. In particular, the CRISPR/Cas9 system was used to induce mutations in the Solyc08g080640 and Solyc10g080210 genes, which encode respectively for the Thaumatin-like protein (TLP) and the Polygalacturonase 2a (PG2a) enzyme, two allergens present in tomato fruit.

Gene-specific sgRNAs were designed and constructed with low off-target scores. The DNA constructs were assembled using Goldenbraid, and components of the CRISPR-Cas9 system were delivered into plant cells via Agrobacterium-mediated transformation. The transformation was performed on cv Moneymaker

tomato plants previously edited by the CRISPR/Cas9 system, which led to the silencing of two genes: the GAME4 gene, involved in the biosynthetic pathway of glycoalkaloids, and Sola l 4, one of the major tomato allergens.

Regeneration of plant tissues rapidly occurred on selective media containing the antibiotic kanamycin, and Cas9 and sgRNA expression cassettes were stably integrated into the plant genome. Finally, transformed or regenerated plants with the desired modifications were identified by polymerase chain reaction (PCR) genotyping and confirmed by sequencing.

Eight edited plants for the TLP and PG2a genes were obtained by the CRISPR/Cas9 system and transferred to the greenhouse. Lines derived from those initial edited plants will be obtained and characterized by sequencing around the edited locus, off target and by detailed phenotypic evaluation (absence of the targeted gene expression) and susceptibility to biotic and abiotic stresses.

Eventually, our approach will allow us to obtain tomato lines with low or no allergenic proteins. In addition, our results will show that it is possible to target and stack different genes using CRISPR/Cas9 genome editing.